GONOCOCCUS TYPES 2

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In a previous paper [1] it is shown that absorption experiments threw 85 strains of gonococci (1-85) into 6 distinct heterologous types. The agglutinins produced by strains of one type could not be absorbed by strains of another. In 5 the agglutinins were always bound by strains belonging to the same type: that is, the strains that absorbed the agglutinins of one absorbed as well the agglutinins of another. The antigen complex of the strains forming a type seemed to have a similar constitution, and when injected into animals stimulated the production of like agglutinins. Strains forming type 2, however, varied in their agglutinogenic and absorptive capacity, for, while a number of strains bound the agglutinins produced by some strains completely and constantly, the binding capacity in others was more or less limited and variable, and in a few entirely absent. On closer study the 36 strains of this type grouped themselves into 4 fairly distinct and characteristic subtypes, the *a, b, c,* and *d* races: strains 42-64 form the *a,* 66 and 67 the *b,* 65, 69-74 the *c,* and 68, 75-78, the *d* race. The members of these 4 races will also be referred to as the *a, b, c,* and *d* strains. The methods used here were the same as in the first paper, and what was said there in regard to them applies here also.

Strain 42 stimulated the formation of agglutinins the affinity of which was limited to the *a* strains. Table 1 gives the absorption results of the serum produced by injections with this strain. One c.c. of a 1:100 dilution of this serum was absorbed by the growth of a large slant each of type 2 strains, and then tested for agglutinins with the immunizing strain. Each *a* strain removed the agglutinins completely, but the *b* strains and the 2 available *d* strains did not. So marked was the avidity of the *a* strains for these agglutinins that even the much larger amount present in 1 c.c. of 1:20 dilutions was altogether bound by 8 of the *a* strains, and only 2 left small traces. The *b* and *d* strains on the other hand failed to extract any in a tenfold, a 1:200, dilution.

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[1] Hermanies: J. Infect. Dis, 1921, 28, p. 133.

At the time of these tests the *c* and some of the *d* strains were not at hand. Meanwhile, the serum deteriorated, lost its potency and its specificity to such a degree that absorption tests gave unsatisfactory results. Another rabbit was immunized with the same strain. But the serum produced was of a rather low titer. Table 2 gives the results. Again, of the six *a* strains employed, all deprived the serum of its agglutinins, the five *c* and the *d* 67 removed small amounts compared with the other *d* and the control. This rabbit's serum seemed slightly to differ from the previous one in that some of its agglutinins had an affinity for strains of the other races. They apparently were of different nature and present only in relatively small amounts. The bulk of the agglutinins produced by this second rabbit on stimulation with strain 42 could not be absorbed by the *c* and *d* strains although the *a* strains bound them completely. The absorbing strains evidently had an antigen constituent in common with strain 42, and since it differentiated them from the other strains of type 2, and characterized them as members of the *a* race, it was called the *a* agglutinogen, and its antibodies the *a* agglutinins. The latter failed to combine with the members of the other races, because these strains were deficient in the *a* constituent or had it only in small amounts.

The results were different when a serum was produced by injections with *a* 63. A 1:100 dilution was used in table 3. After absorption by the growth of one large slant of strains of type 2, the serum was tested with strain 63. The *a* strains, the *b* 66, and the *c* 65 extracted the agglutinins completely. *B* 67 and the other *c* strains usually left traces unbound. Their absorptive capacities varied: sometimes they absorbed altogether and sometimes they left small amounts. Three of the *d* strains were able to remove moderate amounts of these agglutinins, two failed entirely.

In table 4 a 1:25 dilution was treated in the same way. Strains 65 and 66, that in the previous higher dilution deprived the serum of all the agglutinins, were no longer able to absorb them completely. Only traces remained unbound by strain 65, while strains 66, 73 and 74 left moderate, and 70-72 large, amounts unabsorbed. The absorption by strains 67 and 76 was so limited that enough agglutinins were left in a 1:800 dilution to cause a partial agglutination of strain 63. When this serum was diluted 1:50 and the amount of the agglutinins reduced to half, *c* 65 extracted them completely, *b* 66 and *c* 73 left traces, and the other *c* strains left small and moderate amounts. *B* 67, and the *d* strain

TABLE 1

STRAIN 42, SERUM 1 C c OF 1: 100 DILUTION ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 42

Gonococcal Strains	Serum Dilutions			Immunologic Races of Type 2
	1:200	1:800	1:1600	
42, 47, 55-58	0	0	0	a
49, 60, 61, 64	0	0	0	a
66	+++	+++	+	b
67	+++	+++	+++	b
76, 75	+++	+++	++	d
Control	+++	+++	++	

In this and the succeeding tables the degree of agglutination is indicated by the number of plus signs, +++ being complete, ++ moderate, + partial but definite; 0 means absence of agglutination.

In the final arrangement of the tables strains giving identical results were treated collectively.

The results of the more decisive dilutions only were recorded in these tables.

TABLE 2

STRAIN 42, SERUM 1 C c OF 1: 50 DILUTION ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 42

Gonococcal Strains	Serum Dilutions			Immunologic Races of Type 2
	1:100	1:200	1:400	
43-46, 50-52	0	0	0	a
69-74	+++	+++	0	c
76	+++	+++	0	d
75	+++	+++	+	d
Control	+++	+++	+	

TABLE 3

STRAIN 63, SERUM 1 C c OF 1: 100 DILUTION ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 63

Gonococcal Strains	Serum Dilutions				Immunologic Races of Type 2
	1:200	1:400	1:800	1:1600	
42-64	0	0	0	0	a
66	0	0	0	0	b
67	++	0	0	0	b
65*	0	0	0	0	c
69-74	++	0	0	0	c
68, 75, 76	+++	++	0	0	d
77, 78	+++	+++	+++	+++	d
Control	+++	+++	+++	+++	

* In the first paper strain 65 by mistake was placed in the *a* instead of the *c* race.

76 again left the largest amounts unabsorbed. In a dilution of 1:200 the absorption by *d* 68, 75, and 76 was rather limited, by 77 and 78 absent. The *c* strains removed the agglutinins completely, while *b* 67 left traces unbound.

These absorption tests with varying dilutions of the serum produced by immunization with strain 63 thus revealed a marked difference in the binding capacity of type 2 strains for the agglutinins induced with this *a* strain. The agglutinogen that gave rise to this type of agglutinins formed a relatively large and constant constituent in the *a* strains. It must have differed from the *a* agglutinogen, since it was present in the *b* and *c* strains, and even in some of the *d* strains. In 77 and 78 it either was entirely absent or formed such a small compotent in the antigen complex as to have no binding ability for the corresponding agglutinins. The prevalence of this agglutinogen in most of the strains of this type justifies one in considering it a characteristic of the entire type. It was called the *x* agglutinogen and its antibodies the *x* agglutinins.

These absorption experiments furthermore separated strains 42-64 from the other strains of type 2. Strain 42 induced the *a* agglutinins, the affinity of which was limited to the *a* strains, because they alone had the *a* agglutinogen. The *x* agglutinins, incited by the *x* agglutinogen of strain 63, were also absorbed in much larger amounts by the *a* strains. They had, however, a wider range of action and could combine with the majority of the other strains, since the antigen constituent stimulating their production was present in them, though in smaller and variable amounts, and very likely in a somewhat altered configuration. The *a* strains thus revealed the presence of two large constituents in their antigen complex, the *a* and *x* agglutinogens, which enabled them to absorb both types of agglutinins. The members of the other races, though deficient in the *a* agglutinogen, had the *x* agglutinogen as a constituent in their antigen make-up.

The serums of these two strains not only differentiated the *a* strains as members of a distinct and characteristic race, but the *x* agglutinins of strain 63 also distinguished the *d* strains from the two other races as having for these agglutinins either a very limited or an entirely absent binding capacity. The *b* and *c* strains, though varying in their affinities for the *x* agglutinins, could not be separated from one another.

A serum produced by immunization with strain 67 discriminated strains 66 and 67 from strains 65, and 69-74. In table 5 this serum was diluted 1:200, and 1 c.c. of this dilution was absorbed by the growth of

TABLE 4

STRAIN 63, SERUM 1 C C OF 1: 25 DILUTION ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 63

Gonococcal Strains	Serum Dilutions						Immunologic Races of Type 2
	1:50	1:100	1:200	1:400	1:800	1:1600	
42, 45, 46, 48-50	0	0	0	0	0	0	a
51, 53-57, 64	0	0	0	0	0	0	a
66	+++	+++	++	0	0	0	b
67	+++	+++	+++	++	+	0	b
65	++	0	0	0	0	0	c
73, 74	+++	++	+	0	0	0	c
69-72	+++	+++	+++	++	0	0	c
76	+++	+++	+++	+++	++	0	d
77, 78	+++	+++	+++	+++	+++	++	d
Control	+++	+++	+++	+++	+++	++	

TABLE 5

STRAIN 67, SERUM (OLD) 1 C C OF 1: 200 DILUTION ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 67

Gonococcal Strains	Serum Dilutions				Immunologic Races of Type 2
	1:400	1:800	1:1600	1:3200	
46, 48, 49, 51	+++	+++	+++	++	a
53, 57, 63	+++	+++	+++	++	a
56, 45, 47	+++	+++	+++	+	a
43, 44, 50, 52	+++	+++	++	0	a
66	+	0	0	0	b
67	0	0	0	0	b
71, 74	+++	+++	++	0	c
69, 70, 72, 73	+++	+++	+++	+	c
75, 76	+++	+++	++	+	d
Control	+++	+++	+++	+++	

TABLE 6

STRAIN 67, SERUM (OLD) 1 C C OF 1: 400 DILUTION ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 67

Gonococcal Strains	Serum Dilutions					Immunologic Types
	1:800	1:1600	1:3200	1:6400	1:12800	
47, 49, 50, 52, 55, 60	+++	++	++	+	0	2a
51, 52, 56-58, 63, 64	+++	++	+	0	0	2a
66	+	0	0	0	0	2b
67	0	0	0	0	0	2b
75	++	++	+	0	0	2d
76	+++	+++	++	+	0	2d
2, 6, 10	+++	+++	++	++	0	1
1, 3, 4, 7-9	+++	+++	+++	++	+	1
14, 23-29	+++	+++	+++	++	+	1
82, 83	+++	+++	++	+	0	4
84	+++	+++	++	+	0	5
85	+++	+++	++	++	0	6
Control	+++	+++	+++	++	+	

one large slant of each of the strains of type 2. When tested for the presence of agglutinins with the immunizing strain only the two *b* strains (66 and 67) removed the agglutinins; the other strains generally failed. Diluted 1:400, and treated like the previous dilution, the results obtained were similar. They are recorded in table 6. Some of the *a* and *d* strains appeared to have extracted part of the agglutinins, others left them fully intact, as did the strains of the other types.

Additional absorptions in dilutions of 1:100, 1:200, and 1:250 were made. With strain 67 the absorption was always complete; strain 66 either bound the agglutinins entirely or left only traces. Some of the *a* strains at one time would deprive the serum of considerable quantities of agglutinins, at another not remove any, even in higher dilutions. Generally their absorptive ability was changeable and limited, or wholly absent. When a 1:200 dilution, after absorption, was tested for the presence of agglutinins with strain 66, the results in the main agreed with those of strain 67 (table 7).

TABLE 7

STRAIN 67, SERUM (OLD) 1 C C OF 1:200 DILUTIONS ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 66

Gonococcal Strains	Serum Dilutions				Immunologic Races of Type 2
	1:400	1:800	1:1600	1:3200	
43, 44, 57	+++	+++	+++	+	a
47-50, 56	+++	+++	++	+	a
52, 53, 46	+++	+++	++	0	a
59, 63	+++	++	0	0	a
66, 67	0	0	0	0	b
71, 73	+++	+++	++	0	c
69, 70, 72, 74	+++	+++	++	+	c
68	+++	++	0	0	d
77	+++	+++	++	+	d
Control	+++	++	++	++	

Strain 67 thus stimulated the formation of agglutinins the affinity of which was more or less restricted to strains 66 and 67. Since the constituent that incited these agglutinins characterized the members of the *b* race, it was called the *b* agglutinogen and its specific antibodies the *b* agglutinins. Of the other strains the majority were either altogether in want of this constituent or had it in such small quantity as not to combine with perceptible amounts, and if they did combine, this capacity was only passing.

Entirely different facts emerged, when a 1:100 dilution after absorption was tested for the presence of agglutinins with the *a* strain 47. Most of the *a*, *b*, and *c* strains absorbed the agglutinins for this strain completely, and only a few left small amounts unbound. The *d* strains

again either entirely failed or extracted slight amounts. None of the heterologous strains revealed binding capacity.

When a 1:100 dilution, after absorption by strains of type 2, was tested with the *c* strain 71, the *a*, *b*, and *c* strains again absorbed the agglutinins completely. Of the two *d* strains used, 76 absorbed but 77 failed.

The fact that the *a*, *b*, and *c* strains were able to remove the agglutinins for strains 47 and 71 proved that strain 67 had given rise to another type of agglutinins, a type which agglutinated these strains and was absorbed by them. Strain 67 thus stimulated the production of two varieties of agglutinins, the specific or race, and the more general or type, agglutinins. The latter, and the *x* agglutinins of strain 63 were apparently of similar constitution, and were actuated by a compotent analogous to the *x* agglutinogen in strain 63. Strain 67 had such a constituent. It was able to absorb the *x* agglutinins of strain 63 in moderate amounts. The lesser and quite limited binding capacity was explained as due to smaller amounts of the *x* entity in this strain. These more general agglutinins no doubt were incited by it. They differed, however, from the *x* agglutinins of strain 63 in that they were absorbed in nearly the same amounts by the *a*, *b*, and *c* strains, while the latter were bound in much larger quantities by the *a* than by the *b* and *c* strains. The members of the last two races besides disclosed a more varied binding ability for the *x* agglutinins of strain 63 than for those of strain 67. It looks as though the *x* agglutinogen of strain 67 had a different configuration from the one of strain 63. In *b* 66 and *c* 65 this compotent was more like that in the *a* strains, for they combined with the *x* agglutinins of strain 63 nearly as well as did the *a* strains themselves. Only in the lower dilutions they failed, while in the higher the binding was always complete. Their affinity for these agglutinins was certainly greater than that of the other *b* and *c* strains. The *c* strains generally bound larger amounts of the *x* agglutinins of strain 63 than did the *b* strain 67. Their *x* agglutinogen differed from the one of the *a* strains, but had not changed so extensively as that of strain 67, and thus seemed to have an intermediate position. The *x* agglutinogen of the *a* strains appeared more alien than the same constituent of the *c* strains to the *x* agglutinins of strain 67. Both races nevertheless removed equal amounts of these agglutinins. Apparently it was present in larger quantities in the *a* than in the *c* strains. These facts justify the assumption that the differences in the avidity for the *x* agglutinins of strain 63

were not solely due to varied amounts of the *x* agglutinogen in these races, but somewhat to differences in the structure of this antigen constituent.

The bulk of the *b* agglutinogens in the two *b* strains did not coincide. Strain 67 contained relatively larger amounts of the *b* constituent, since it always was able to bind the *b* agglutinins completely, while strain 66 at times left traces unabsorbed. And the *x* constituent, as already mentioned, was, while similar, not identical in the two strains. The relative proportion of these two constituents was subject to quite marked and sudden changes, as was shown about half a year later with another rabbit. The serum produced with strain 67, was of approximately the same titer as the previous one. When 1 c.c. of a 1-25 dilution was absorbed by strains of type 2, the *a* and *c* strains were able to bind the agglutinins partially (table 9). Now either the *x* agglutinogen which strain 67 had in common with the *a* and *c* races had in the meanwhile increased and stimulated a larger production of the *x* agglutinins, or that particular rabbit responded more to the stimulation of the *x* than the *b* compotent. This serum, no doubt, had larger amounts of the *x* agglutinins, since with it in a much higher dilution there was very little absorption of the agglutinins by the *a* and *c* strains (table 5). In strain 66 the relative proportion of the two compotents (the *b* and *x* agglutinogens) had swung decidedly in favor of the *x* constituent. It absorbed the *b* agglutinins with difficulty. The *b* agglutinogen had meanwhile receded to such an extent that its absorptive capacity for the *b* agglutinins was much reduced. This was confirmed by the fact that, when after absorption by type 2 strains it was used in testing for the presence of agglutinins, most of the *a* and *c* strains extracted the agglutinins for it (table 10). These same strains were unable to absorb the agglutinins of the older serum for strain 66 (table 7). The *b* agglutinins remaining after absorption by these strains were sufficient to induce clumping of strain 66, because it possessed enough of the *b* agglutinogen. With the new serum after such an absorption there was no agglutination. Apparently the *b* agglutinogen had so receded that the comparatively small amounts of the *b* agglutinins in a dilution of 1:200 were no longer able to exert their action on this strain. However, the *b* agglutinogen had not disappeared entirely, the strain still retaining some *b* characteristics. Thus when the serum was only diluted 1:100, strains 66 and 67 absorbed completely, the *a* and *c* strains generally left small amounts unbound.

TABLE 8

STRAIN 67, SERUM (OLD) 1 C c OF 1: 100 DILUTIONS ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 47

Gonococcal Strains	Serum Dilutions				Immunologic Types
	1:200	1:400	1:800	1:1600	
42, 48, 50-53	0	0	0	0	2a
56, 57, 63, 64	0	0	0	0	2a
54, 60	++	0	0	0	2a
66	+	0	0	0	2b
67	0	0	0	0	2b
69, 70, 72, 74	0	0	0	0	2c
71	++	0	0	0	2c
73	+++	+	0	0	2c
75	+++	+++	+	0	2d
76	++	++	++	+	2d
77	+++	+++	+++	++	2d
1, 5, 7, 38-40	+++	+++	++	+	1
3, 4, 6, 9-12, 14	+++	+++	+++	++	1
16, 17, 19, 22, 41	+++	+++	+++	++	1
81	+++	+++	+++	+	3
83	+++	+++	+++	0	4
84	+++	+++	+++	+	5
85	+++	+++	+++	++	6
Control	+++	+++	+++	++	

TABLE 9

STRAIN 67 SERUM (NEW) 1 C c OF 1: 25 DILUTION ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 67

Gonococcal Strains	Serum Dilutions					Immunologic Races of Type 2
	1:50	1:100	1:200	1:400	1:800	
54, 57, 64	+++	+++	+++	+	0	a
46, 48, 51, 63	+++	+++	++	+	0	a
42, 45, 50, 53	+++	+++	++	0	0	a
66	+++	+++	+	0	0	b
67	0	0	0	0	0	b
70, 72-74	+++	+++	+	0	0	c
65	+++	+++	++	0	0	c
69, 71	+++	+++	+++	+	0	c
68	+++	++	+	0	0	d
76-78	+++	+++	+++	++	+	d
Control	+++	+++	+++	+++	++	

TABLE 10

STRAIN 67, SERUM (NEW) 1 C c OF 1: 200 DILUTION ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 66

Gonococcal Strains	Serum Dilutions				Immunologic Races of Type 2
	1:400	1:800	1:1600	1:3200	
42, 46, 49, 50, 51	0	0	0	0	a
54, 57, 59, 63, 64	0	0	0	0	a
48	+	0	0	0	a
53	++	+	0	0	a
66-67	0	0	0	0	b
69-72, 74, 65	0	0	0	0	c
73	+	0	0	0	c
68	0	0	0	0	d
76	+++	+++	0	0	d
77, 78	+++	+++	+	0	d
Control	+++	+++	++	+	

When the same dilution after absorption was tested for the presence of agglutinins with the *c* strain 71, the *a, b,* and *c* strains absorbed the agglutinins completely, showing that the *x* agglutinins could be handled by these strains. The small *b* compotent could still absorb some of the *b* agglutinins; if their amounts were large it absorbed enough to induce flocculation of strain 66. In the presence of smaller amounts the absorption was not sufficient for its agglutination.

The members of the *a, b, c* races were linked to each other by the *x* agglutinins, and their relationship in the type was thus established directly. The *d* strains on the other hand showed little or no affinity for these agglutinins, and their relation in type 2 remained doubtful. Strains 68, 75, and 76 generally bound small amounts though their absorptive capacity varied much. Strain 76 once absorbed the *x* agglutinins of strain 67 for strain 71, and strain 68 absorbed them completely for strain 66 and nearly so for 71. At times the absorption was quite marked, at other times it was absent. Strains 77 and 78 generally failed, and the slight occasional absorption would not justify placing them in this type.

TABLE 11

STRAIN 69, SERUM 1 C c OF 1:100 DILUTION ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 69

Gonococcal Strains	Serum Dilutions					Immunologic Types
	1:200	1:400	1:800	1:1600	1:3200	
48-51, 53, 54	+++	+++	+++	++	+	2a
42, 44, 45, 47, 63	+++	+++	+++	++	+	2a
46, 64	+++	+++	+++	++	0	2a
66, 67	+++	+++	+++	++	+	2b
65	+++	+++	+++	++	0	2c
69-74	0	0	0	0	0	2c
76-78	++	0	0	0	0	2d
3, 16, 41	+++	+++	+++	+	0	1
1, 2, 4, 5, 7, 10	+++	+++	+++	++	+	1
11, 14, 20-22, 39	+++	+++	+++	++	+	1
32, 34, 36, 40	+++	+++	+++	++	+	1
79-81	+++	+++	+++	++	+	3
83	+++	+++	+++	++	+	4
84	+++	+++	+++	++	+	5
85	+++	+++	+++	++	+	6
Control	+++	+++	+++	++	+	

Absorption tests with a serum produced by the *c* strain 69 established them, however, as a distinct race of type 2. Table 11 gives the results. One c.c. of 1:100 dilution of this serum was absorbed by the growth of one large slant of the following strains, and then tested for agglutinins with strain 69. None of the *a* and *b* strains of types 2 were able to absorb any. The *c* strains, however, with one exception absorbed

them completely, and the *d* strains left only traces. The strains of the other types bound none of the agglutinins. In a dilution of 1:200 the *a* and *b* strains again failed, but strain 67 absorbed partially. *C* 65, failing in the previous lower dilution, now completely bound the reduced amount of the agglutinins. The *d* strains removed all the agglutinins. When a 1:100 dilution after absorption was tested with strain 76, every one of the *c* strains absorbed the agglutinins, the two *d* strains again leaving traces. *B* 67 was able to bind fairly large amounts of agglutinins for this strain (table 12). Note that even a few of the *a* strains appear to have extracted some. In the last two tables *d* 77 was used in testing for the presence of agglutinins after absorption by the various strains. The results were clear-cut. When the serum was diluted 1:100, as in table 13, all the *c* and *d* strains absorbed the agglutinins completely, but the *a* and *b* strains left them wholly intact. Even in a 1:400 dilution, as in table 14, they failed to bind any agglutinins, in which they did not differ from strains of the heterologous types.

These absorption results show that strain 69 produced agglutinins which could not be bound by any *a* strains. *B* 66 was never able to absorb, though *b* 67 absorbed partially for 69 in a 1:200 dilution, and nearly completely for 76 in a 1:100 dilution. The agglutinins were without difficulty bound by all *d* strains. They must have had a compotent similar in constitution to that in the *c* strains, and present in relatively the same proportions. This same compotent was either entirely absent or present in exceeding small amounts in most *a* and *b* strains. To some extent, however, it entered into the composition of strain 67, since on two occasions it showed a tendency to bind these agglutinins. The quite extensive though limited absorptive capacity of the *c* strains for the *x* agglutinins of the serum induced by 63 and 67 showed that all had the *x* agglutinogen as a constituent in their antigen complex. In strain 69, however, this compotent did not stimulate the formation of agglutinins. Several attempts were made to determine their presence. When a 1:100 dilution after absorption was tested with the *a* and *b* strains the controls failed to agglutinate. Since these strains were agglutinated with difficulty and only in low dilutions, the amount of the *x* agglutinins, if at all present, was limited indeed. It was the *c* agglutinogen that the *d* strains shared in common with the *c* strains. This enabled them to absorb the *c* agglutinins and indirectly linked them to this type.

TABLE 12

STRAIN 69, SERUM 1 Cc OF 1:100 DILUTION ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 76

Gonococcal Strains	Serum Dilutions					Immunologic Races of Type 2
	1:200	1:400	1:1600	1:3200	1:6400	
46, 50, 63	+++	+++	+++	+++	++	a
49, 51, 53	+++	+++	++	++	+	a
44, 48, 54. 57	+++	+++	++	++	0	a
45, 64	+++	+++	+	0	0	a
66	+++	+++	+++	++	++	b
67	++	+	0	0	0	b
65, 69-74	0	0	0	0	0	c
77, 78	++	0	0	0	0	d
Control	+++	+++	+++	+++	++	

TABLE 13

STRAIN 69, SERUM 1 Cc OF 1:100 DILUTION ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 77

Gonococcal Strains	Serum Dilutions			Immunologic Types
	1:200	1:3200	1:6400	
42, 46, 49, 50	+++	+++	+++	2a
51, 53, 57, 63	+++	+++	+++	2a
45, 44, 48, 54, 64	+++	+++	+++	2a
66, 67	+++	+++	+++	2b
65, 69-74	0	0	0	2c
68, 76-78	0	0	0	2d
7, 16	+++	+++	+++	1
15	+++	+++	+++	1
84	+++	+++	++	4
Control	+++	+++	+++	

The heavy type indicates that after absorption by these strains there was partial agglutination of the testing strain in a 1:12,800 dilution.

TABLE 14

STRAIN 69, SERUM 1 Cc OF 1:400 DILUTION ABSORBED BY THE GROWTH OF ONE LARGE SLANT OF THE FOLLOWING STRAINS AND TESTED WITH STRAIN 77

Gonococcal Strains	Serum Dilutions			Immunologic Types
	1:1600	1:3200	1:6400	
42, 44-46, 48-51	+++	+++	+++	2a
53, 54, 57, 64	+++	+++	+++	2a
63	+++	+++	0	2a
66, 67	+++	+++	+++	2b
22	+++	+++	0	1
1, 4, 5, 10-12, 41	+++	+++	+++	1
14-16, 19, 42	+++	+++	+++	1
3, 7, 34, 36, 39, 40	+++	+++	+++	1
81	+++	+++	+++	3
83	+++	+++	+++	4
84	+++	+++	+++	5
85	+++	+++	+++	6
Control	+++	+++	+++	

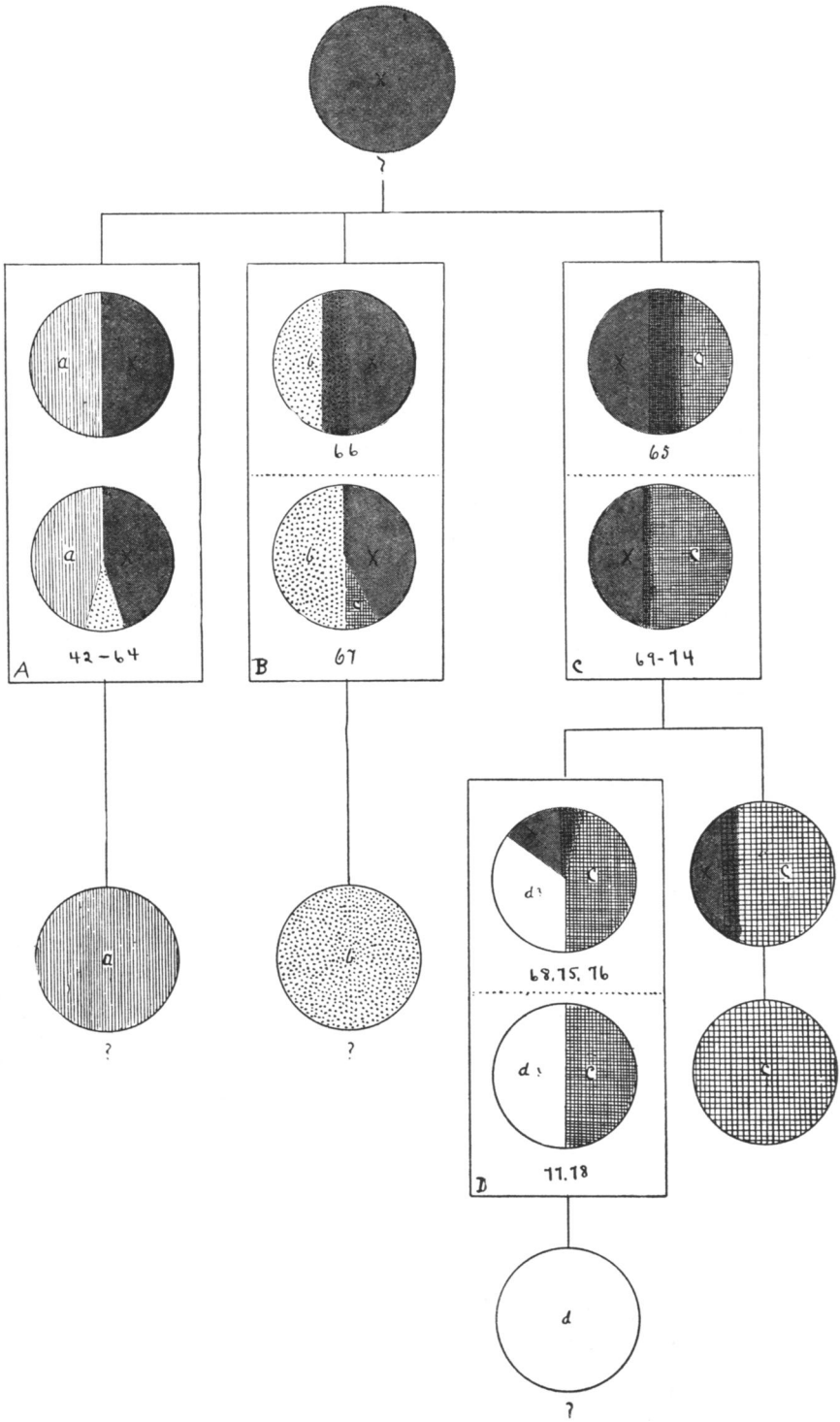
DISCUSSION

The antigen complexes of the 4 races of type 2, their interrelationships, and their probable path of evolution are shown graphically in the genealogic table:

The circles included in the *A, B, C,* and *D* squares represent the antigen of the *a, b, c,* and *d* races. The *a* strains brought to light two large and permanent compotents—the *a* and *x* agglutinogens—the relative proportions of which, while fairly constant, were nevertheless, subject to slight variations, variations more pronounced in some strains than in others. In strain 42 the *a* agglutinogen stimulated the formation of the *a* agglutinins, the affinity of which was limited to the *a* strains, since the *a* moiety was their specific characteristic. The representatives of the other races failed to combine with them, because they lacked this entity. In strain 63 the *x* agglutinogen was active and incited the *x* agglutinin which had a wider but varied range of action. Their avidity for the *a* strains was marked, more restricted and variable for the *b* and *c* strains, quite limited or absent for the *d* strains. This variation in the binding capacity can be explained as due to differences in amounts and in the constitution of the *x* constituent in the strains of the 4 races, and was tentatively indicated by the diversity in size and shading of this compotent in the diagram. Both strains had the 2 compotents, the *a* and *x* agglutinogens being alternately active and passive.

The antigen of the 2 members of the *b* race also had 2 main constituents, the *b* and *x* agglutinogens, and when strain 67 was used for immunization both actuated their respective agglutinins. The affinity of the specific race agglutinins was restricted to the *b* strains, the more general *x* agglutinins being bound with comparative ease and in approximate equal amounts by the *a, b,* and *c* strains. In this they differed from the *x* agglutinins of strain 63, which were absorbed in varying amounts by these strains.

These two *x* agglutinins were of similar but not identical constitution and differed in their molecular arrangement. They offer proof that the *x* agglutinogen of strain 67 had a different configuration than the same moiety in strain 63. This is in accord with the assumption that this compotent had a slightly different and varying constitution in the several races. Thus the variable binding capacity of the several races and even of the individual strains was at least partially due to the differences in the configuration of the *x* agglutinogens and not merely to variations in amounts.



Genealogy of four races of type 2.

The *c* strains had the *x* agglutinogen, since they were able to bind both varieties of the *x* agglutinins. The quantity and configuration apparently differed from the same entity in the other races and varied even in the individual strains as indicated by their somewhat fluctuating and unequal binding capacity. They had their specific race constituent, the *c* agglutinogen. In strain 69 it alone was active and led to the formation of the *c* agglutinins. The latter with one exception had no affinity for the *a* and *b* strains. They were, however, absorbed constantly in large amounts by the *c* and *d* strains. The *d* strains thus shared this constituent in common with the *c* strains. It entered, however, into the make-up of the antigen of strain 67, since this strain was occasionally able to combine with fairly large amounts of the *c* agglutinins.

D strains differed from *c* strains in the slighter avidity for *c* agglutinins, and in the limited or absent binding capacity for both *x* agglutinins. They were represented as composed of two families which differed from each other in that the antigen complex of the one had a small amount of the *x* agglutinogen while the antigen complex of the other had none. They evidently had another undetermined constituent the nature of which was not revealed—no serum having been produced. Notwithstanding, this constituent was assumed and regarded as specific for these strains, and was called the *d* agglutinogen. The *c* agglutinogen may, however, have undergone alteration of structure and thus despite their presence in larger amounts in these strains, the latter had a lesser affinity for the *c* agglutinins than the *c* strains proper. This possibility is visualized in the last two figures on the right. In that case they should be regarded rather as variants of the *c* race, the agglutinogen of which either had receded still farther or was lost entirely.

The *x* agglutinogen, excepting the two *d* strains, was a common constituent of all the races of this type, and is no doubt primary and oldest. Evidently at one time it was the sole compotent and is as such represented in the hypothetical type from which these several races of type 2 differentiated themselves. Owing to the inherent tendency of living matter to vary, some strains succeeded, either by new combinations or by altering the configuration of some of the *x* agglutinogens and by changing them into certain ways, in elaborating new structures. Thus, if a certain modification proved to be advantageous to the species the strains exhibiting this variation were favored. Starting from this advantage, the variation continued in that particular direction until a

new constituent was evolved. It may have been in an attempt to overcome and evade the antibodies developed by the host against its specific antigen that certain strains differentiated this new compotent. Their advantage once revealed, they became exalted and increased in amounts, the old meanwhile receding. Some strains may have acquired this through gradual and progressive alteration. In others the change may have been abrupt. Again in some strains this variation and differentiation led to the formation of the *a* agglutinogen, in others it resulted in the *b* and *c* moieties. At least these 3 and possibly 4 variations had definitely established themselves in the 36 strains of this type. The primary *x* antigen not only receded, but also underwent slight modification which was along different lines in several races. In the 22 strains forming the *a* race, the *a* and *x* constituents appeared to be fairly balanced. Evidently all the gonococci in these strains had both constituents. The newly acquired racial character had stabilized and firmly established itself. The temporary fluctuations in the individual strains apparently were due to transient changes in the relative proportion of these 2 compotents. The same conditions seem to prevail in the majority of the *b* and *c* strains. The more marked and sudden changes in *b* 66 and in *c* 65 call for some other explanation. Thus in 66 the *b* moiety must have receded to very small proportions, since its binding capacity for the *b* agglutinins became very limited, and only when they were present in large amounts was it able to combine with sufficient quantities to agglutinate the strain. Apparently this strain was composed of two varieties of organisms. Some had acquired the *b* agglutinogen and consequently had both the racial and the type agglutinogens in their antigen complex. Others were deficient in the specific *b* constituent, and their antigen had merely the *x* compotent, thus approaching the assumed primary hypothetical type composition from which these races supposedly developed. If by chance the bulk of the culture consisted of the latter, the strain extracted little or no racial agglutinins. The degree of absorption to a certain extent thus depended on the relative proportion of the two types of organism having the *xb* and the *x* structure. At an earlier date these two types were fairly balanced, though subject to daily variations, one in excess of the other. Thus one day the strain was able to bind the *b* agglutinins of strain 67 completely and on the next day left traces. Later the organisms with the *x* constituents decidedly dominated. Its binding ability for the *b* agglutinins was very limited. It shows how by still

farther reduction of the organisms having the racial *xb* antigen complex this strain may have reverted to the hypothetical *x* type. It affords a good example of how pure chance is a factor in the development of the immunologic types from preexisting races, or the reverse.

The fact that strain 67 on several occasions was able to bind fairly large amounts of the *c* agglutinins, though generally failing to absorb any of them, can best be explained on the same supposition. No doubt there were some gonococci that had the *c* agglutinogen in their antigen complex. If thus it chanced that in a certain culture they were present in great numbers, the strain was able to absorb large amounts of the *c* agglutinins. Some of the *a* strains had cocci that had the *b* agglutinogen in their antigen composition, because occasionally some of them were able to absorb fairly large amounts of the *b* agglutinins of strain 67. Even the *c* strain 65 must have had organisms with the simple *x* antigen composition, since once it failed to absorb any of the *c* agglutinins.

The three *d* strains that still had the *x* agglutinogen in their antigen complex were quite erratic in their ability to bind the *x* agglutinins. Generally they absorbed little but sometimes a strain was able to bind large quantities. It apparently was composed of two types of organisms, one having the *xc*, the other the *cd* antigen combination, and the natural fluctuation in the relative proportion of these two organisms explained their varying absorptive abilities. The *d* strains 77 and 78 no doubt had lost the *xc* compotent entirely.

The hypothetical antigen composition below the 4 races indicates how by a farther shifting and elimination of the *x* constituent 4 distinct types can be evolved. Strains having these antigen compositions would have no relation whatsoever with each other and would constitute 4 separate and distinct immunologic types. It shows how immunologic species may eventually be formed from races by the gradual elimination of an old and common constituent that linked these races into a single type. That such a final elimination is possible was shown by strains 77 and 78 of the *d* race. Had it not been for the *c* agglutinins which they were able to bind they would have to be excluded from type 2, and would then constitute a new type. Judging from analogy the 6 distinct and separate gonococcic types discussed in the previous paper may have sometime been merely races of one or two types. In the course of evolution the common connecting bond was eliminated and the races became new species. As the process of variation is still going on, and will continue as long as their living conditions are secured, there is no

limit to farther differentiation. A single clear-cut type may by molecular rearrangement acquire new antigen constituents and split into several races. Finally, by elimination these may differentiate into species.

This marked lability and variation in quantity and constitution of the various compotents constituting the antigen complexes of the races of type 2 show that a strain is potentially able to revert to the original type or differentiate into variants definite and characteristic enough to constitute fairly distinct races. Not all the compotents present in an antigen stimulate the formation of agglutinins: in some strains one, and in others a different agglutinogen actuates its specific agglutinins. All this complicates the grouping of strains having such variable and complex antigen composition, necessitating a large number of serums for the establishment of their interrelationships within a single type. Thus type 2 is unique and distinct from the other 5 types, no strains of other types being able to absorb its agglutinins.